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Numerical determination of the competitive isotherm of enantiomers

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Abstract

A numerical method was developed and used to determine adsorption isotherms in chromatography. The numerical parameters of an isotherm model were derived from the recorded band profiles of the racemic mixture of the 1-phenyl-1-propanol enantiomers, by means of a nonlinear least-squares method. We used the equilibrium-dispersive model of chromatography with several isotherm models. The numerical constants of the isotherm models were tuned so that the calculated and the measured band profiles match as much as possible. We show that this numerical inverse method can be applied even without the knowledge of the individual band profile of the pure enantiomers. The isotherms determined from the—usually unresolved—overloaded band profiles matched extremely well the isotherms determined by frontal analysis. Several isotherm models were used and tested—such as Langmuir, biLangmuir, Tóth, Langmuir–Freundlich. The best-fit isotherm was selected by means of statistical evaluation of the results.

Keywords: Adsorption isotherms; Isotherm models; Enantiomer separation; 1-Phenyl-1-propanol

1. Introduction

Because the behavior at high concentrations of most characteristics of separation systems is nonlinear, preparative separations are very difficult to design. Since the nonlinear nature of preparative chromatography is governed by that of the equilibrium isotherms, the experimental determination of adsorption isotherms is of utmost importance in the design of new methods in preparative chromatography. When developing a process-scale separation, the scale-up from analytical to large-scale is a very demanding task. The isotherm determination cannot be avoided if one wants this tedious and expensive task to be completed in an economic, optimal manner.

Several dynamic methods are available to determine equilibrium isotherms by chromatography. Unfortunately, most of these methods may be applied only for the determination of single component isotherms. The most popular methods are the frontal analysis (FA), the elution by characteristic point (ECP), the frontal analysis by characteristic point (FACP), and the perturbation (injection on a plateau, PM) methods [1]. Of these methods, FA affords

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equilibrium isotherm data points of which several dozens must be collected covering a sufficiently wide concentration range, from the quasi-linear initial part of the isotherm to the region in which the amount adsorbed is a significant fraction of the saturation capacity. It is simple and accurate provided that the mass transfer kinetics is reasonably fast. It has also been applied to the determination of competitive isotherms in ternary mixtures [2,3]. By their very principle, the ECP and FACP methods cannot be applied to determine competitive isotherms, and, even for single-component isotherms, these methods should only be used with columns of high efficiency ($N > 5000$ theoretical plates) because they neglect axial dispersion [4]. In contrast with the FA method, the perturbation method does not supply isotherm data points but allows the derivation of the best estimates of the coefficients of an isotherm equation from the set of elution times of perturbations generated on a series of concentration plateaus recorded at increasing concentrations.

These methods can be grouped into two categories, pulse methods (ECP and FACP) that derive isotherm data from the elution chromatogram of a single, large size pulse and plateau methods (FA and PM) that derive these data from events taking place on concentration pulses. Obviously, the methods of the first type are more parsimonious with chemicals than the latter ones since with plateau methods, the determination of each isotherm point requires a separate experiment and this demands large amounts of sample and solvent. Accordingly, we need a pulse method that would be reasonably accurate and could be applied to mixtures of compounds.

The direct problem of chromatography consists of calculating the band profiles of the components involved knowing their equilibrium isotherms. The inverse problem consists in deriving the isotherm from recorded band profiles. Fundamentally, a chromatographic method of determination of equilibrium isotherms is, in this case, a method that attempts to generate chromatographic signals under such conditions where it is easy to derive an accurate solution of the inverse problem of chromatography. The isotherm data are acquired by performing series of chromatographic measurements in which the concentrations of the components are adjusted systematically. In FA, the simplicity of the boundary condition (the Riemann condition) results in the equilibrium constant being simply related to the elution time of the concentration shock. Since the actual shock layer that takes place in the experiment propagates at the same velocity as the shock [1], there is no model error. In ECP, the relationship between the velocity associated with a concentration and this concentration is used to derive the differential of the isotherm from the rear profile of a high concentration band. In this case, however, there is a model error since the concept of a velocity associated with a concentration is a property of the hyperbolic equation of the ideal model of chromatography but does not apply to the parabolic equation of the equilibrium-dispersive model. For our purpose, any method that could derive the equilibrium isotherm from accurately measured elution profiles would be a pulse method of determination of isotherms.

Experimentally, the requirements are that the boundary condition, i.e. the injection profile should be accurately known. This condition should approximate as much as possible a rectangle but, in practice, significant deviations from this profile are almost always observed [5]. The elution profile should be accurately known. Elution profiles in chromatography are usually plots of the UV absorbance of the eluent versus time. The absorbance must be transformed into concentration. Most UV detectors are very sensitive and are not linear in the concentration range investigated in isotherm measurements. For multicomponent isotherm determinations, a multicomponent calibration would be needed. It seems far simpler to collect the elution fractions and analyze them [6–8]. Then the simultaneous solutions of the chromatographic problem are known for the different components of the system. The competitive isotherms are determined by inversion of the solution, which requires (1) assuming an isotherm model; (2) determining initial values of its parameters; (3) calculating the corresponding band profiles; and (4) adjusting the parameters to minimize the difference between experimental and calculated profiles. The choice of the proper isotherm model is facilitated by the fact that elution chromatography is a differential method. The shape of the recorded band profile informs on the shape of the isotherm. From the shape

of an overloaded band, an experienced chromatographer can judge whether a convex, a concave, or an S-shaped isotherm is to be assumed. Yet, there are many isotherm equations for convex upward isotherms and the shape of the band depends critically on the isotherm equation.

This approach is a pulse method like the ECP and FACP methods but has the advantage that band profile calculations can be made using the equilibrium-dispersive model or even the POR model to take into account the effects of axial dispersion and mass transfer resistances on these profiles. So, the inaccuracy arising from model error can be considerably reduced. However, as with the perturbation method, a major shortcoming of this method is that an isotherm model should a priori be assumed. We can go around this disadvantage only by trying several properly chosen isotherm models and choosing the one that gives the best matching band profiles.

This approach can be regarded as a parameter identification problem of a chromatographic model [9]. Dose et al. determined the equilibrium isotherms of *N*-benzoyl-(D,L)-alanine and *N*-benzoyl-(D,L)-phenylalanine on immobilized bovine serum albumin from the peak shapes of single components [10]. They used a modified simplex algorithm to find the best parameters of biLangmuir isotherms and found a good agreement with the isotherms determined with frontal analysis. James and Sepúlveda developed a more sophisticated algorithm for the estimation of the isotherm parameters by the inverse method [9,11]. They used the conjugate gradient algorithm for the minimization of the objective function that—besides the conventional least squares—took into account the difference between the first moments of the peaks, as well. This algorithm was applied for the estimation of the competitive biLangmuir and Moreau isotherms of Ketoprofen enantiomers on a Chiracel OJ column, as well as of the competitive Langmuir isotherm of benzyl alcohol and 2-phenylethanol on a C_{18} column from individual band profiles [12]. The competitive isotherms estimated with the inverse method agreed well with the data obtained with conventional methods. Zhang et al. applied the inverse problem to isotherm determination with computer-simulated chromatograms and analyzed the effect of experimental errors on the accuracy of the isotherm parameters [13]. Antos et al. used the Marquardt algorithm to fit the band profiles in order to estimate single component isotherms in a normal phase system [14].

We refer to this method of isotherm determination as the *inverse method* hereafter. This inverse method is becoming popular as a quick procedure for the estimation of the competitive isotherms necessary for designing simulated moving bed (SMB) separations. SMB is a field of large-scale separations where the operational range cannot be designed conveniently without knowing the competitive isotherms of the feed components. Juza applied the inverse method in simulated moving bed separations (SMB). The isotherms of cycloheptanone and cyclopentanone were determined on an analytical column containing the same silica gel stationary phase as the SMB system [15]. The isotherms of the same compounds on silica were determined in a solvent gradient SMB process by Antos and Seidel-Morgenstern using the inverse method [16]. The competitive isotherms of the enantiomers of Fenoprofen on a Chiracel OJ stationary phase were identified by Ching et al. to set up an SMB process [17]. It should be emphasized that care should be applied to satisfy the requirement of the method by acquiring accurate band profiles under well-defined experimental conditions and by carefully selecting an appropriate set of equilibrium isotherms. Experimental isotherm data often fit approximately to the ubiquitous Langmuir model, they rarely fit well to it [1].

The aim of this study is to demonstrate that competitive isotherms can be estimated with a good accuracy using the inverse method from the band profiles of a racemic mixture obtained in the overloaded elution mode, without measuring any of the single-component chromatograms. To validate our isotherm determination procedure, the chromatograms of 1-phenyl-1-propanol obtained on a microbore system [18] are employed and the isotherms calculated here are compared with the isotherms obtained by frontal analysis. The competitive adsorption isotherms of the racemic mixture of 1-phenyl-1-propanol enantiomers on cellulose tribenzoate have recently been studied by frontal analysis on a widebore [19] and on a microbore column [18]. We will take advantage of a considerable simplification of the method that is available in the case of

enantiomers. The UV-spectra of two enantiomers are identical. Accordingly, the concentration profile of the two components can be derived from the conventional chromatograms through a simple calibration which, even if the detector response is not linear, can be obtained rapidly and derived from simple measurements made with either enantiomer or with the racemic mixture. As we will show, the competitive isotherms of the two enantiomers can be derived accurately from a single chromatogram obtained with as large a sample as possible. For enantiomer separations, the methods that can determine competitive isotherms from racemic mixtures are very beneficial, because the pure enantiomers are usually expensive and very difficult to obtain.

2. Theory

The equilibrium-dispersive model of chromatography can be employed for the modeling of many nonlinear separations [1]. In this model we assume instantaneous equilibrium between the stationary and the mobile phases, and use an apparent dispersion term to account for both the axial dispersion and the finite rate of the mass transfer kinetics. The following mass balance equation is written for each component of the sample:

$$\frac{\partial C_i(z,t)}{\partial t} + F\frac{\partial q_i(z,t)}{\partial t} + u\frac{\partial C_i(z,t)}{\partial z} = D_a\frac{\partial^2 C_i(z,t)}{\partial z^2} \tag{1}$$

where C_i and q_i are the concentrations of component i in the mobile and the stationary phases, respectively; z is the length, t the time, u the mobile phase linear velocity, and F the phase ratio, $(1-\varepsilon_t)/\varepsilon_t$, where ε_t is the total porosity of the column. D_a is the apparent dispersion coefficient that can be calculated from the number of theoretical plates (N) determined by an analytical injection:

$$D_a = \frac{uL}{2N} \tag{2}$$

where L is the column length.

The apparent dispersion coefficient describes zone spreading in linear chromatography. This phenomenon is mainly governed by axial dispersion in the mobile phase and by nonequilibrium effects (i.e. the consequence of a finite rate of mass transfer kinetics). The band spreading observed in preparative chromatography is far more extensive than it is in linear chromatography. It is predominantly caused by the consequences of the nonlinear thermodynamics, i.e. the concentration dependence of the velocity associated to each concentration. When the mass transfer kinetics is fast, the influence of the apparent axial dispersion is small or moderate and results in a mere correction to the band profile predicted by thermodynamics alone. Then, it is computationally very convenient to assume identical apparent diffusion coefficients for the two enantiomers.

The initial condition $C_i(z,\ 0) = 0$ states that at $t = 0$ the column is equilibrated with the pure mobile phase. As a first approach, we assume that the sample is introduced into the column as a rectangular pulse.[3] The concentration of the component i in the sample is C_i^0, and the duration of the sample injection is t_p.

The Danckwerts boundary conditions describe the feed flux at the column inlet and outlet, respectively:

$$uC_i^0 = uC_i(0,t) - D_a\frac{\partial C_i(z,t)}{\partial z}\bigg|_{z=0} \tag{3}$$

$$D_a\frac{\partial C_i(z,t)}{\partial z}\bigg|_{z=L} = 0 \tag{4}$$

Because the chromatographic columns used in actual practice have a high efficiency, the classical Danckwerts boundary conditions can be written simply for each component as:

$$C_i(0,t) = C_i^0 \qquad 0 < t \le t_p \tag{5}$$

The system of mass balance equations with the proper isotherm equations is to be integrated numerically to obtain the concentration profiles at the column outlet.

[3] In most practical applications this assumption is unrealistic and cannot be used. The actual injection profile, which differs from a rectangular pulse must be taken into account. The actual injection profile should then be determined and used in the model, particularly when the injection time and/or retention time is small [5].

3. Experimental

An Agilent 1100 Series Capillary Chromatograph (Agilent Technologies, Palo Alto, CA, USA), equipped with a micro diode array detector, a flow splitter and a computer data station, was used for all experiments. The instrumental set-up is described in detail elsewhere [18]. The mobile phase was a solution of *n*-hexane and 2-propanol (97:3, v/v). Hexane and 2-propanol were HPLC grade solvents from Fisher Scientific (Fair Lawn, NJ, USA). All the solvents were filtered (0.2 μm Gelman Sciences, Ann Arbor, MI, USA) before use. 1,3,5-Tri-*tert.*-butylbenzene (TTB), used as the nonretained marker, was purchased from Aldrich (Milwaukee, WI, USA). The racemic mixture of 1-phenyl-1-propanol, also from Aldrich, was previously purified in our laboratory.

A 15×0.1 cm stainless steel column packed with Chiracel OB (cellulose tribenzoate coated on a silica support; Daicel, Tokyo, Japan) was used for all the measurements. The column was packed by Micro-Tech Scientific (Sunnyvale, CA, USA). The average particle size of the packing material was 20 μm. The total porosity, measured by injecting TTB, was $\varepsilon_t = 0.795$. The column holdup time was 18.7 min. The efficiency of the column at a flow rate of 5 μl/min was about $N = 1200$ theoretical plates.

For the determination of the competitive isotherms, four injections of a racemic mixture (10.02, 19.35, 34.81, and 45.98 μg) were used.

4. Calculations

For the calculation of the individual profiles, the system of two partial differential equations is solved using a finite difference scheme written for Eq. (1) with $D_a = 0$ (ideal model). The values of the time and length increments of the integration are chosen such that the numerical dispersion will exactly be equal to the desired apparent dispersion [1]. For the numerical integration, a modified Rouchon (finite difference) algorithm was used, which ignores the empty sections of the (z, t) plane and accordingly markedly speeds up the calculations [20].

The isotherm parameters were determined with the following algorithm.

(i) An isotherm model was selected and initial estimates were determined for its numerical parameters.
(ii) Band profiles of the racemic mixture were calculated with the above described algorithm.
(iii) The measured and calculated band profiles were compared by evaluating the following objective function:

$$\min \sum_i r_i^2 = \min \sum_i (C_i^{\text{sim}} - C_i^{\text{meas}})^2 \qquad (6)$$

where C_i^{sim} and C_i^{meas} are the calculated and the measured concentrations at point i and r_i is their difference.
(iv) The isotherm parameters were changed to minimize the objective function, using the following two nonlinear least squares algorithms: the super modified downhill simplex search [21] and the Levenberg–Marquardt steepest descent method [22].

5. Results and discussion

5.1. Fitting the Langmuir isotherm

The obvious first choice to model non-linear isotherms in chromatography is to employ the competitive Langmuir isotherm. The competitive Langmuir isotherm for either component is given as:

$$q_i = \frac{q_s b_i C_i}{1 + b_1 C_1 + b_2 C_2} \qquad (7)$$

where q_s is the saturation capacity and b is the equilibrium constant or distribution coefficient.

When we assume that both enantiomers have identical saturation capacities, three parameters remain to fit: q_s, b_1, and b_2. Very good initial estimates of two of the isotherm parameters can be derived from an analytical injection of the racemic mixture. When the retention times and the hold-up time of the column are available, we obtain an excellent first guess for the $a_i = q_s b_i$ parameters of the competitive Langmuir isotherm:

$$t_{R,i} = t_0(1 + k_i') = t_0(1 + F q_s b_i) = t_0(1 + F a_i) \qquad (8)$$

where k_i' is the retention factor of component i. In our numerical calculations, we first set the a_i param-

eters of the competitive Langmuir isotherm at these estimates and fitted only the b_i terms. That fitting poses no difficulty at all. Then, the results of this preliminary fit were used to fit simultaneously all the isotherm parameters.

We used the four overloaded chromatograms in different combinations in order to obtain the isotherm parameters. In the first set-up we fitted the isotherm to each individual chromatogram, although we knew in advance that we could not expect a good estimate of the coefficients b_i from the injections made at low loading factors. The best-fit chromatograms are plotted in Fig. 1, the numerical result of the fitting is summarized in Table 1.

The results suggest that the saturation capacity is consistently underestimated if the loading factor is too low. In that case, the curvature of the isotherm (b_iC_i) is not sufficient to perform a reliable fit, the results of which can be extrapolated to higher concentrations (which is often the implicit purpose of determining isotherm parameters). The determination of the saturation capacity is always an extrapolation.

Table 1
Isotherm parameters obtained with the competitive Langmuir model

Sample size (μg)	q_s	b_1	b_2	FSSR
10.02	37.86	0.1766	0.2179	0.0074
19.35	48.78	0.1362	0.1662	0.0474
34.81	60.77	0.1063	0.1285	0.189
45.98	65.28	0.0996	0.1200	0.351
All	64.24	0.1008	0.1219	2.561
Frontal	83.00	0.0779	0.0906	
$c < 2.3$ g/l	78.44	0.0828	0.0977	
$c < 1.3$ g/l	70.84	0.0916	0.1111	

So, the more limited the concentration range sampled by the bands, the larger the error we may expect for the extrapolated saturation capacity. Furthermore, any model and/or experimental error will immensely affect that extrapolation.

Obviously, it is not only the inverse method that suffers from this error due to unjustified extrapolation. The frontal analysis method also does. In the last three lines of Table 1, we show the isotherm

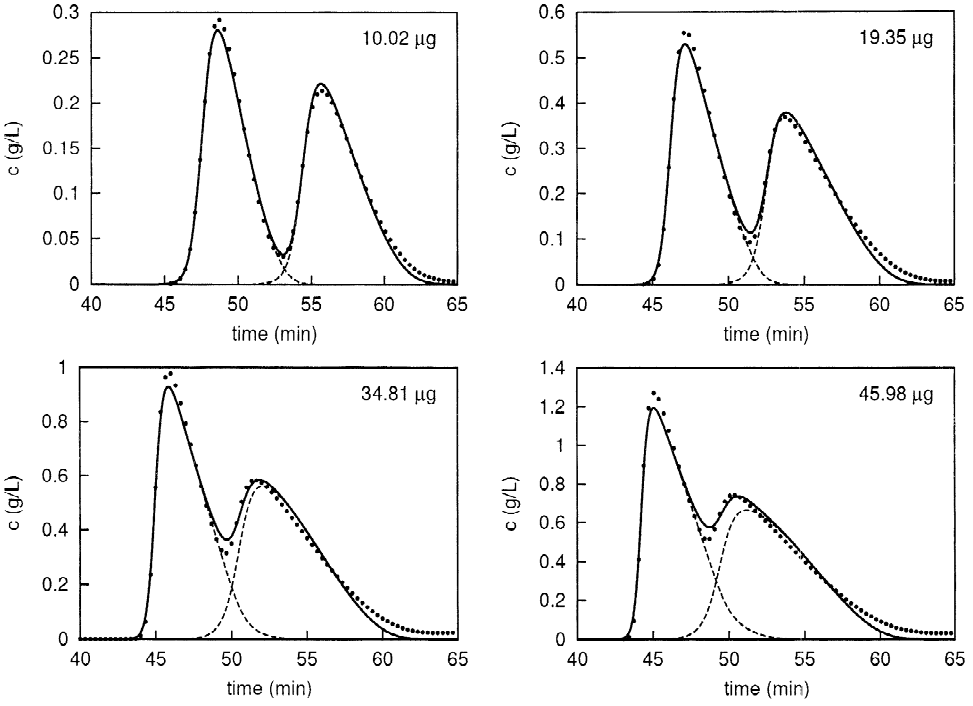

Fig. 1. Best-fit overloaded profiles—using competitive Langmuir isotherm model—determined by individual fit of each chromatogram. The points represent the experimental chromatograms, the lines are the simulated elution profiles.

parameters obtained by fitting the competitive Langmuir model to the data determined by frontal analysis. The two concentration subranges correspond to the maximum injected concentration (2.3 g/l) and to the maximum elution concentration (1.3 g/l) of the band in the case of the highest loading factor. Again the same trend of the estimated saturation capacity is observed.

Fig. 2 shows the band profiles that we obtained when all four chromatograms were fitted simultaneously. There is a systematic deviation between the calculated and measured profiles on the tailing part of the more retained enantiomer, and that difference is enhanced at small loading factor. This discrepancy was already seen in Fig. 1 but to a lesser degree. It is most probably caused by a model error, i.e. the Langmuir model might not model well enough the adsorption behavior of the separation system studied.

In Table 1 (line 5) we can see that the low concentration injections have no significant effect on the estimated isotherm parameters. The numerical isotherm parameters obtained by fitting the four chromatograms together are not significantly different from those derived from the largest injection alone.

A comparison of the data presented in Table 1 confirm that the isotherm parameters obtained with the inverse method are very close to those obtained by frontal analysis, in the range up to the maximum elution concentration. In Fig. 3, the different isotherms obtained are plotted and compared. The symbols represent the experimental data obtained by frontal analysis, the dashed line is the best-fit competitive Langmuir model obtained from these FA data, whereas the solid line is the competitive Langmuir isotherm determined with the inverse method. The agreement is excellent.

Up to now, we have assumed that the saturation capacity was the same for both enantiomers. On the one hand, this assumption is required by the condition for thermodynamical consistency of the isotherm [23]. On the other hand, when we determine competitive isotherms by fitting the frontal analysis data for the racemic mixture alone, we cannot

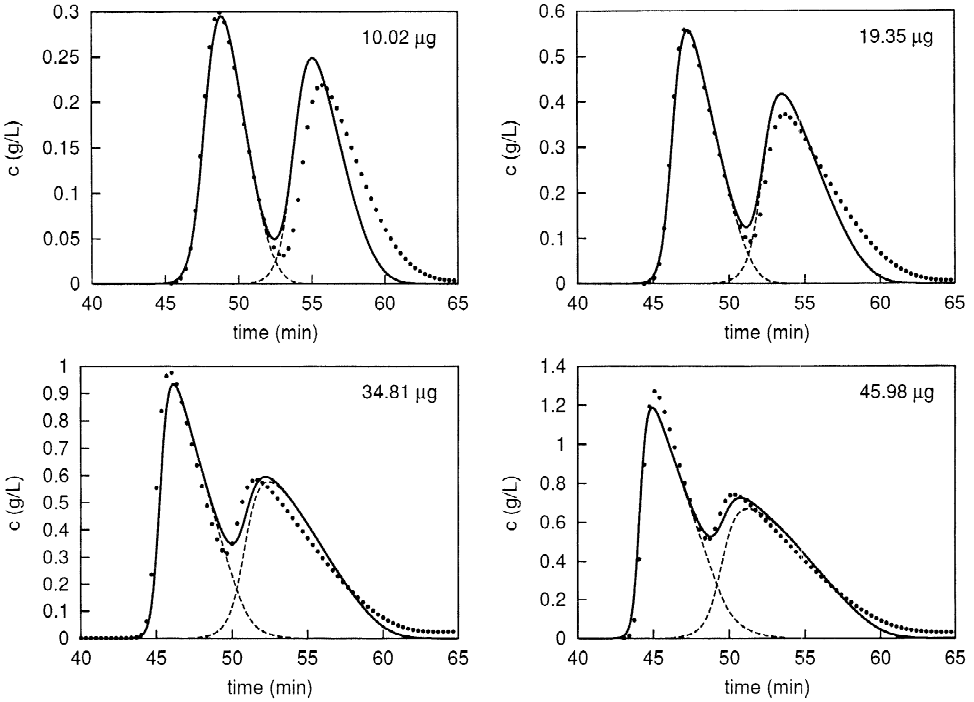

Fig. 2. Best-fit overloaded profiles—using competitive Langmuir isotherm model—determined by the simultaneous fit of all chromatograms.

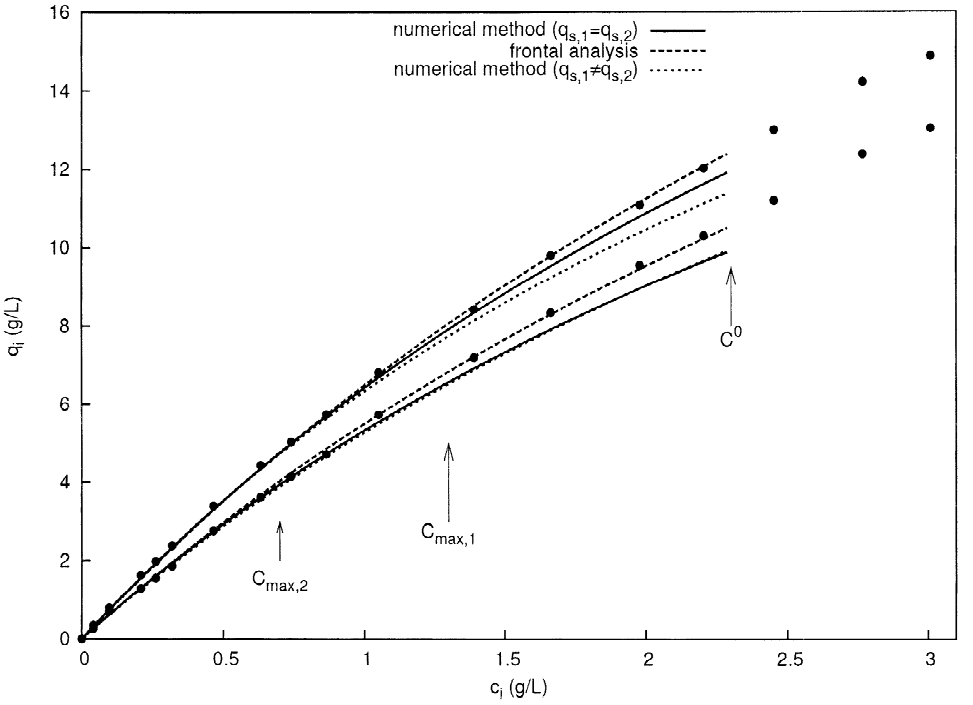

Fig. 3. Comparison of the competitive isotherms obtained by frontal analysis (symbols), and by the inverse numerical method assuming the Langmuir model. $C_{max,i}$ (see the band last profile in Fig. 2) indicate the maximum elution concentration of component i; C^0 is the concentration injected.

eliminate that restriction. Since we use only a racemic mixture to determine the isotherm from the whole [C_1, C_2] plane, we determine the competitive isotherm along the diagonal of that plane only, where $C_1 = C_2$. Thus, the isotherm model that we can fit has the following limited form:

$$q_i = \frac{q_s b_i C_i}{1 + (b_1 + b_2)C_i} \tag{9}$$

Fitting this isotherm to the frontal analysis data, allows the estimate of no more than three parameters: $q_s b_1$, $q_s b_2$, and $b_1 + b_2$.

When we use the inverse method, although this method is applied to the chromatograms of samples of the racemic mixture only, this constraint is lifted because the two enantiomers migrate along the column at different velocities and adsorb at different positions along the column with quite different relative mobile phase concentrations. Therefore, with the inverse method, we sample a large fraction of the [C_1, C_2] plane. This is illustrated in Fig. 4 where each concentration pair is plotted as the band migrates along the column. A large number of different combinations of C_1 and C_2 is involved in the isotherm calculation. The density of the points increases in the neighborhood of the concentration distribution at the column outlet, which is indicated by the solid lines in the bottom left corner of the figure. This concentration distribution forecasts that the isotherm determined with the inverse method will be most accurate for concentrations below the maximum elution concentration.

Calculating the values of the four parameters of the competitive Langmuir model that best account for the chromatogram with the largest loading factor gave the following results: $q_1 = 77.41$, $b_1 = 0.0822$, $q_2 = 52.36$, and $b_2 = 0.1520$ with a final sum of the squares of the residuals FSSR = 0.04517. We can see in Fig. 5 that this model fits much better the original data, particularly for the more retained component. The final sum of the square of residuals is 7.7 times

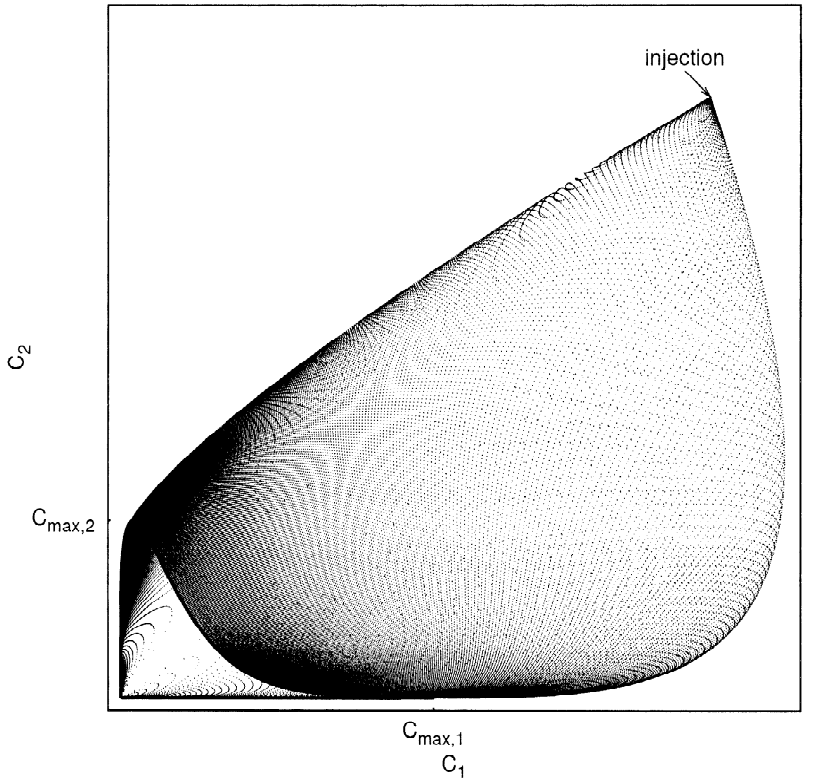

Fig. 4. The mobile phase concentration of the more retained sample component against that of the less retained one for each point of the discrete time and length grid as the bands migrate along the column. The solid line in the bottom left corner indicates the concentration distribution at the end of the column.

smaller after removing the requirement of identical saturation capacity.

Since we used the competitive Langmuir isotherm model with different saturation capacities, we applied the LeVan–Vermeulen approximation in order to restore thermodynamical consistency of the competitive isotherm [24]. It is surprising to see in Fig. 3 that the isotherm for the more retained enantiomer shows a larger deviation from the frontal analysis isotherm although the 4-parameter Langmuir isotherm yielded a much better fit than the 3-parameter model (Fig. 5). When we require that $q_{s,1} = q_{s,2}$, the saturation capacity is estimated from both peaks. When we lift this restriction, $q_{s,2}$ is estimated from the second peak only, whose maximum concentration is much smaller ($\approx 50\%$) than that of the first one. Therefore, the isotherm of the more retained component is valid for a narrower concentration range.

5.2. Fitting the biLangmuir isotherm

When enantiomers are separated on a chiral stationary phase, we expect the stationary phase to be heterogeneous. The nonselective sites retain equally both enantiomers whereas the enantioselective sites interact differently with these two enantiomers, binding them with different energy. To model adsorption on heterogeneous surfaces, several appropriate isotherm models were investigated.

The competitive biLangmuir isotherm model assumes that the surface of the chiral stationary phase contains two different types of sites, the nonselective and the enantioselective sites, either one being homogeneous. The isotherm is written:

$$q_i = \frac{q_{ns} b_{ns} C_i}{1 + b_{ns}(C_1 + C_2)} + \frac{q_s b_i C_i}{1 + b_1 C_1 + b_2 C_2} \qquad (10)$$

where b_{ns} is the equilibrium constant for the ad-

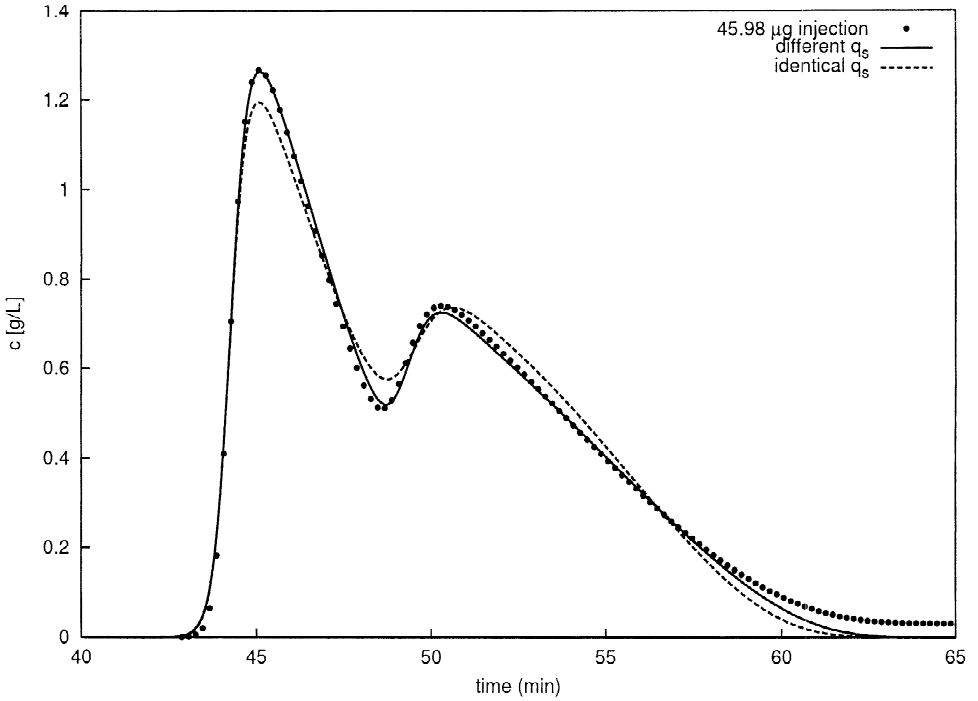

Fig. 5. Chromatograms obtained when fitting the Langmuir isotherm by assuming identical or different saturation capacities for the two enantiomers.

sorption of either enantiomer on the nonselective sites, b_i the equilibrium constant for the adsorption of isomer i on the enantioselective sites, q_{ns} the saturation capacity of the nonselective sites and q_s the saturation capacity of the enantioselective sites, assumed equal for both enantiomers.

We followed the same strategy as with the Langmuir isotherm model. Firstly, we fitted the isotherm parameters for each chromatogram separately. The chromatograms are shown in Fig. 6, and the numerical results are summarized in the first four lines of Table 2. As a general rule, the fit is much better with this isotherm model than with the Langmuir model. In Fig. 7, we present the results of fitting the isotherm parameters using all four chromatograms simultaneously. Again, the agreement between measured and calculated band profiles is very good. Finally, we calculated all the band profiles with the isotherm parameters derived from the one chromatogram obtained with the largest sample size. The results are shown in Fig. 8. The agreement is particularly improved in the case of the chromatogram resulting from small injections, i.e. the biLangmuir model seems to describe the adsorption equilibrium much better for the whole concentration range than the Langmuir isotherm.

The best-fit biLangmuir isotherms are plotted in Fig. 9. The isotherms determined with the inverse method follow the frontal analysis data extremely well, up to the maximum elution concentration.

5.3. Fitting the Tóth and the Langmuir–Freundlich isotherms

Other isotherm models are used to account for the adsorption behavior on the heterogeneous surface of stationary phases. In this study, the Tóth and the Langmuir–Freundlich isotherms were also employed. Both isotherms differ from the Langmuir isotherm only by an exponent, the role of which is to take into account the heterogeneity of the distribution of the sorption energies.

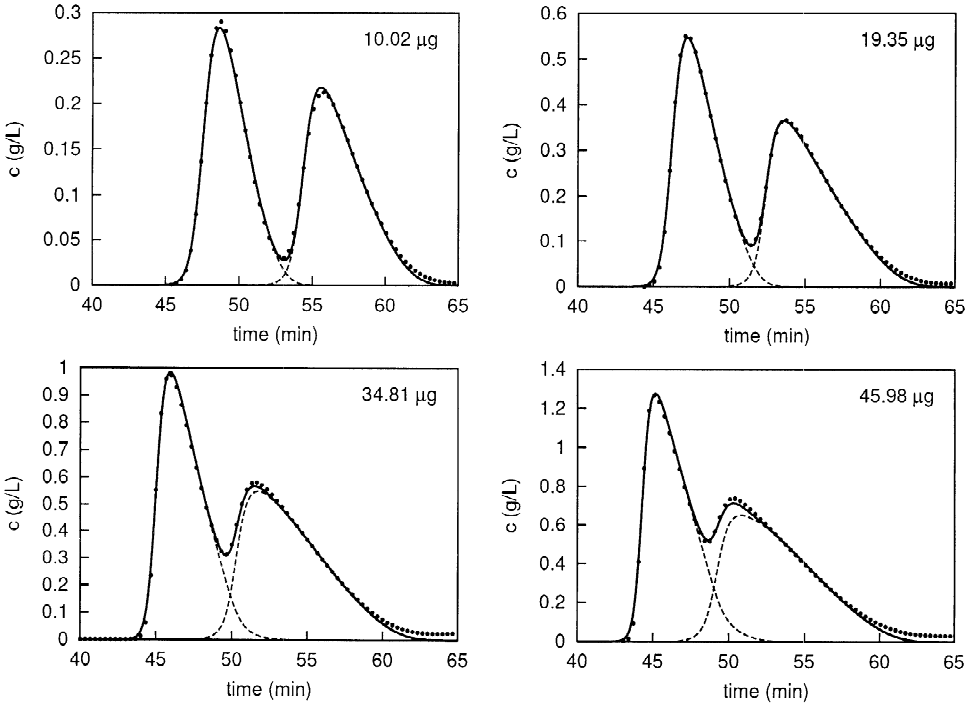

Fig. 6. Best-fit overloaded profiles—using competitive biLangmuir isotherm model—determined by individual fit of each chromatogram.

The competitive Tóth isotherm is written as:

$$q_i = \frac{q_s b_i C_i}{[1 + (b_1 C_1 + b_2 C_2)^{\nu}]^{1/\nu}} \quad (11)$$

where q_s is the saturation capacity, b_i the equilibrium constant for the adsorption of isomer i, and ν is the heterogeneity parameter. For preserving the thermodynamical consistency of the model, the same heterogeneity parameter should be used for both isomers. When the heterogeneity parameter is $\nu = 1$, the Tóth model reduces to the Langmuir isotherm and the surface is homogeneous.

The competitive Langmuir–Freundlich isotherm can also be employed to model adsorption on heterogeneous surfaces:

Table 2
Isotherm parameters obtained with the competitive biLangmuir model

Sample size (µg)	q_{ns}	b_{ns}	q_s	$b_{s,1}$	$b_{s,2}$	FSSR
10.02	40.18	0.1314	5.716	0.1314	0.5405	0.0048
19.35	55.49	0.0932	6.743	0.2053	0.4616	0.0072
34.81	72.76	0.0705	7.307	0.1635	0.3925	0.034
45.98	78.70	0.0655	7.526	0.1577	0.3826	0.082
All	76.20	0.0663	7.432	0.1839	0.4173	1.267
Frontal	98.53	0.0518	7.253	0.1765	0.4157	
$c < 2.3$ g/l	93.49	0.0569	6.372	0.1651	0.4384	
$c < 1.3$ g/l	137.8	0.0370	5.449	0.2320	0.5878	

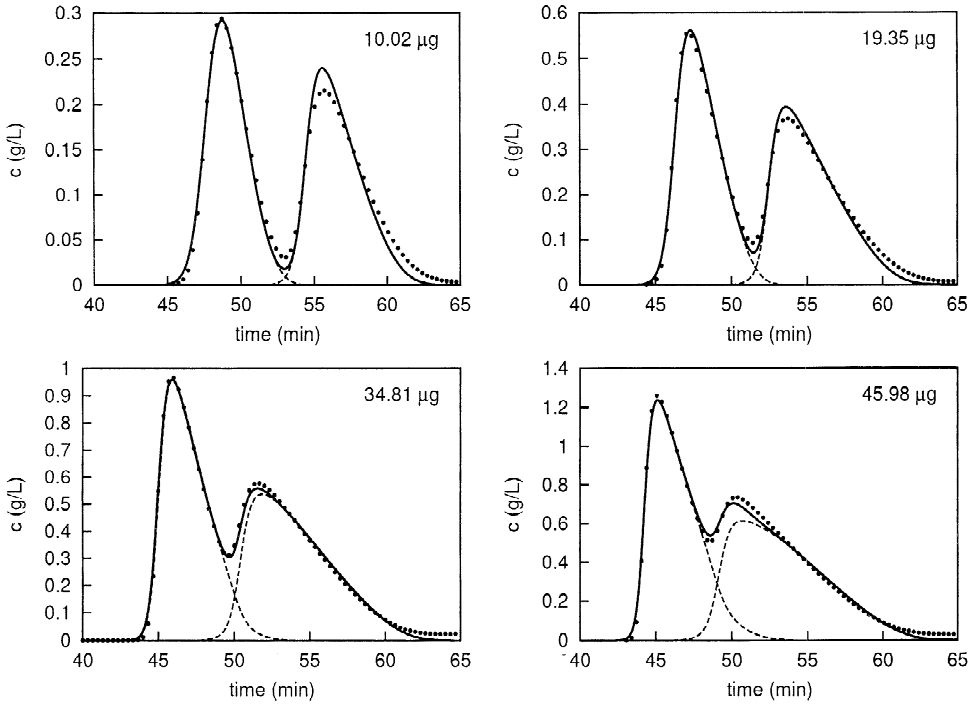

Fig. 7. Best-fit overloaded profiles—using competitive biLangmuir isotherm model—determined by the simultaneous fit of all chromatograms.

$$q_i = \frac{q_s b_i C_i^{\nu_i}}{1 + b_1 C_1^{\nu_1} + b_2 C_2^{\nu_2}} \tag{12}$$

where ν_1 and ν_2 are parameters characterizing the heterogeneity of the adsorption kinetics. Like the Tóth isotherm, the Langmuir–Freundlich isotherm also reduces to the Langmuir isotherm when the heterogeneity parameter becomes $\nu = 1$. Note that the Tóth isotherm has a finite Henry constant, not the Langmuir–Freundlich isotherm, the reason why the former model is often preferred in chromatography.

In Table 3 we report the best values of the isotherm parameters calculated from the chromatogram obtained with the largest injection. The best fit isotherms are plotted in Fig. 10. The Tóth isotherm fits better than the Langmuir but less well than the biLangmuir isotherm. Note that the saturation capacity and the equilibrium constants estimated with the Tóth model are rather different from those obtained with all the other models. The differences in the saturation capacities can be attributed to the value of the parameter ν. Since this parameter is much lower than one in the present case, the curvature of the isotherm is markedly different from that of the other isotherms.

It is important to note that in contrast to the Langmuir or biLangmuir isotherms for which b is the equilibrium constant, in the Tóth model, the equilibrium constant is b^{ν}. The original form of the Tóth isotherm is written as [25]:

$$q = \frac{q_s C}{[1/K + C^{\nu}]^{1/\nu}} \tag{13}$$

where K is the equilibrium constant. Eq. (13) can be written as:

$$q = \frac{q_s bC}{[1 + (bC)^{\nu}]^{1/\nu}} \tag{14}$$

where $b = K^{1/\nu}$. From the data in Table 3, we can calculate $K_1 = b_1^{\nu} = 0.108$ and $K_2 = b_2^{\nu} = 0.121$. These values agree well with the b parameters calculated with the Langmuir and also with the Langmuir–Freundlich models.

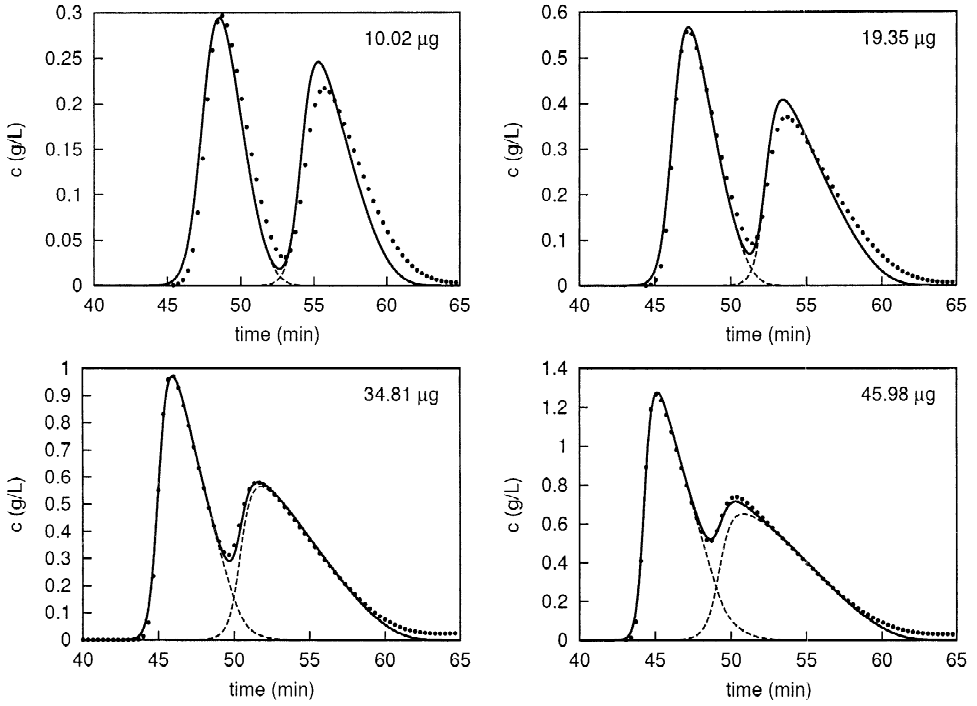

Fig. 8. Overloaded profiles calculated with the biLangmuir isotherm parameters determined by fitting the chromatogram obtained with the 45.98 µg injection.

In the case of the Langmuir–Freundlich isotherm, the parameter ν_1 converged to values very close to one, therefore we fixed its value as $\nu_1 = 1$ and fitted the rest of the parameters only. The value of $\nu_1 = 1$ would indicate a homogeneous retention mechanism for the less retained enantiomers as if it were not retained on the selective sites at all. This isotherm model did not give a better fit than the Langmuir isotherm, in spite of the extra ν_2 parameter involved. This can be explained by the well-known fact that whilst the empirical Langmuir–Freundlich isotherm can well describe the isotherms resulting from adsorption on heterogeneous surfaces, its application in elution chromatography is rather difficult. The slope of the isotherm at the origin approaches infinity when $\nu_i < 1$. This might introduce extremely elongated tailing.

5.4. A comparison with the frontal analysis data

Tables 1–3 show that the numerical values of the isotherm parameters derived from the inverse method differ slightly from those obtained by fitting of the frontal analysis data to the same models. It is also obvious, however, that both the frontal analysis and the inverse method give different numerical values of the parameters when the concentration range within which the corresponding data are acquired is altered. Comparison of the FA data (symbols) and the plotted isotherms reveals that the best isotherm parameters always reflect very well the frontal analysis isotherms up to the maximum elution concentration of the sample.

Fig. 11 shows the domains within which a variation of the isotherm parameters introduces an error smaller than 1% of the stationary phase concentration. The calculations are based on the competitive isotherms currently investigated, in the concentration range $C = 0$ to 3 g/l. As could be expected, the first parameter, $a = q_s b$, of the Langmuir isotherm is the most sensitive, it should not differ by more than 1%. The b parameter, however, is much less sensitive. It can be changed by 10% in the extreme case and the stationary phase concentration calculated with the

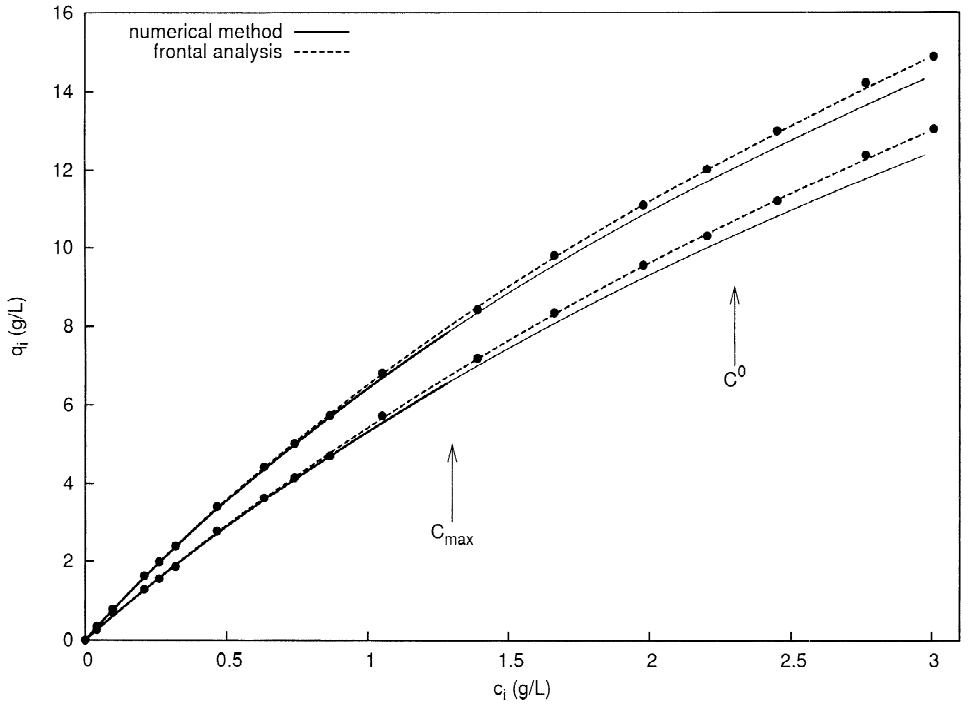

Fig. 9. Comparison of the competitive isotherms obtained by frontal analysis (symbols), and by the inverse numerical method assuming the biLangmuir model. C_{max} indicate the maximum elution concentration of the first component; C^0 is the concentration injected.

isotherm will not vary by more than 1%. This result depends much on the value of bC_{max} and the range would narrow down with the inclusion of the highest concentration profile.

The parameters of the biLangmuir isotherm allow for a larger flexibility. This is due to the fact that this isotherm is the sum of two Langmuir terms. When only the isotherm of the selective or that of the nonselective sites is altered while the other term is untouched, the global effect is smaller. This is particularly true for the term describing the selective sites. Since the abundance of these sites is one order of magnitude smaller than that of the nonselective sites, the b term can be altered by as much as 30% and the total isotherm is changed by less than 1%. Thus, the difference we see in the numerical values of the isotherm parameters is not dramatic.

Table 3
Isotherm parameters obtained with the Tóth and the Langmuir–Freundlich models on the basis of injecting 45.98 μg sample

	Tóth	LaFr
q_s	270.5	65.45
b_1	0.0257	0.0993
b_2	0.0309	0.1193
ν	0.6073	–
ν_1	–	1.0
ν_2	–	0.8567
FSSR	0.1604	0.3506

5.5. Discrimination between isotherm models

The isotherm parameters were determined by a least-squares fitting of the band profiles. The minimization of the squares of the differences between the measured and the simulated chromatograms yields a configuration where the magnitude of the positive and negative errors is the same, therefore their sum is zero. Since $\Sigma r_i = 0$, the variance of the errors is:

$$\sigma_r^2 = \frac{\sum_{i=1}^{n} r_i^2}{n-p} = \frac{\sum_{i=1}^{n} (C_i^{sim} - C_i^{meas})^2}{n-p} \quad (15)$$

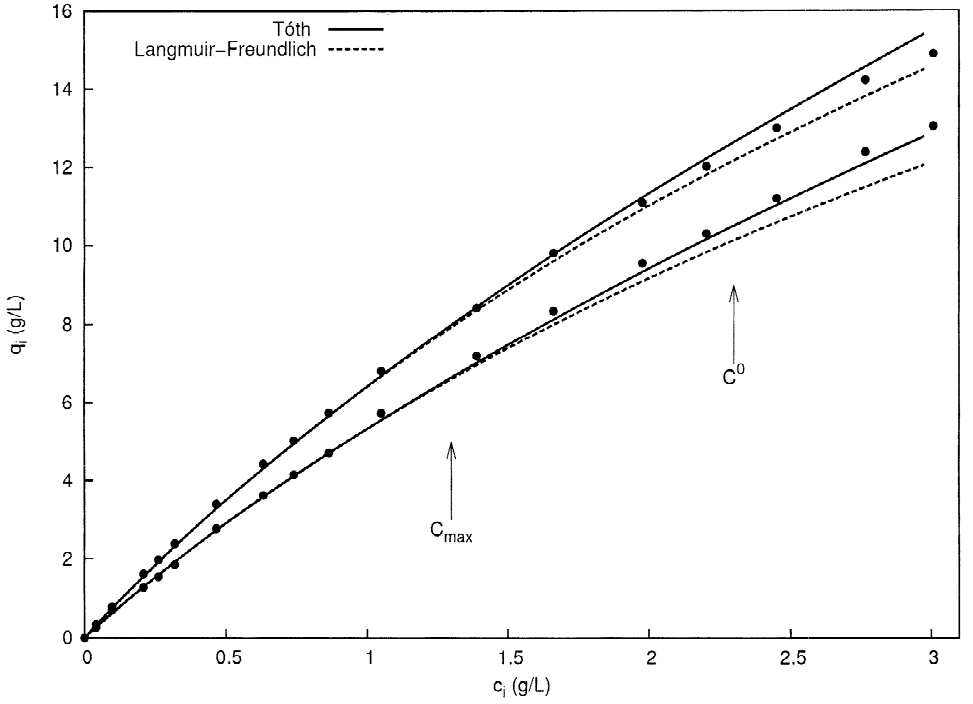

Fig. 10. Comparison of the competitive isotherms obtained by frontal analysis (symbols), and by the inverse numerical method assuming the Tóth and the Langmuir–Freundlich models. C_{max} indicate the maximum elution concentration of the first component; C^0 is the concentration injected.

where p is the number of model parameters fitted. Thus, when we compare the final sum of the squares of the residuals, we compare the variances of the errors. To decide whether or not the two variances significantly differ, the Fisher-test can be applied [26].

Firstly, we checked the normality of the errors with application of the Kolgomorov–Smirnov test as modified by Lilliefors [27]. The original Kolgomorov–Smirnov test can only be applied when the mean and the variance of the distribution are known. When the mean and the variance are estimated from the sample, the modified Kolgomorov–Smirnov test should be employed.

For the normality tests, we calculated an empirical distribution function $F_n(r)$ of the errors and compared it with the normal distribution $F_0(r)$ completely defined by its zero mean and σ_r standard deviation (calculated by Eq. (15)). Our goal was to check the null hypothesis, whether the unknown empirical distribution is identical to $F_0(r)$. The measure of the agreement is the maximum difference between the empirical and the hypothesized distribution functions:

$$D_n = \max_r |F_n(r) - F_0(r)| \tag{16}$$

The test rejects the null hypothesis when $D_n > d_{n,\alpha}$. The critical $d_{n,\alpha}$ values are reported by Lilliefors [27]. For $\alpha = 0.05$, when n is not very small, $d_{n,0.05} = 0.886/\sqrt{n}$ can be used. The empirical and the hypothesized distributions are plotted in Fig. 12, in one instance, that of the comparison of the errors of fitting the 3-parameter and the 4-parameter competitive Langmuir models. The maximum deviation for the 3-parameter Langmuir isotherm was $D_n = 0.0601$ ($n = 75$), whereas for the 4-parameter Langmuir isotherm it was $D_n = 0.0475$ ($n = 40$). Since $d_{75,0.05} = 0.102$ and $d_{40,0.05} = 0.140$, so we cannot reject the null hypothesis in either case. This confirms our assumption that the errors have a normal distribution with a zero mean.

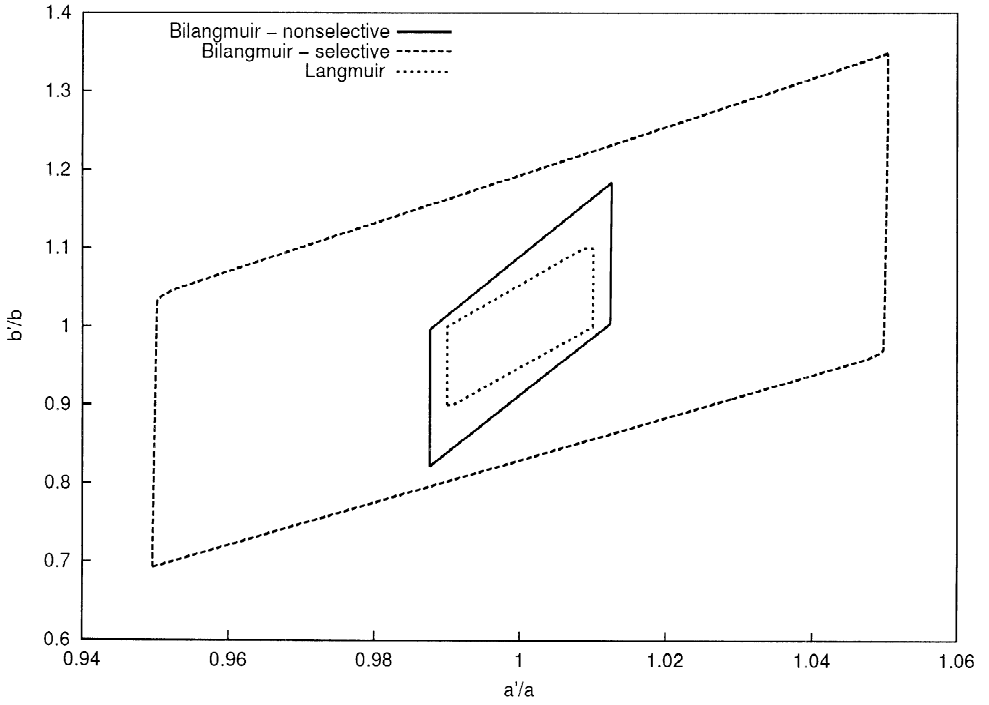

Fig. 11. Domains where the relative error of the isotherm is less than 1%; $a = q_s b$. a'/a and b'/b indicate the relative changes of the isotherm parameters, respectively.

Next, we calculate the F-ratio when comparing the final sum of squares of the residuals for two models as:

$$F = \frac{n - p_2}{n - p_1} \frac{\sum r_{i,1}^2}{\sum r_{i,2}^2} \tag{17}$$

The number of data points in the chromatograms was $n = 320$ and the number of isotherm parameters was $p = 3$ to 5, depending on the model. Thus, as $n \gg p$, the first term on the right-hand-side of Eq. (17) is very close to one and the expression can be simplified as:

$$F = \frac{\sum r_{i,1}^2}{\sum r_{i,2}^2} \tag{18}$$

It is sufficient to compare the ratios of the final sum of squares of the residuals to see whether one model gives a significantly better fit. The critical value of the F-ratio for $\alpha = 0.05$ is $F_{m,m,\alpha} = 1.204$ where $m = n - p = 315$. Since the number of data involved in the fitting is quite large, a 20% improvement of the FSSR is already significant.

When the isotherm models were fitted to the frontal analysis data, the biLangmuir and the Langmuir–Freundlich isotherms fitted equally well, showing no significant difference between these two models. The Langmuir and the Tóth models gave a much worse fit, a nearly fivefold FSSR, but again no difference could be shown between these latter two models [18].

The results obtained in this case with the inverse method agree well with the conclusion of the initial investigation of the frontal analysis data. They confirm that the model that best accounts for the experimental measurements is the biLangmuir isotherm model and that the worst model is the Langmuir equation. The inverse method suggests, however, that the Langmuir–Freundlich isotherm does not fit any better than the Langmuir isotherm. The F-ratio for this isotherm pair is merely $F = 1.001$.

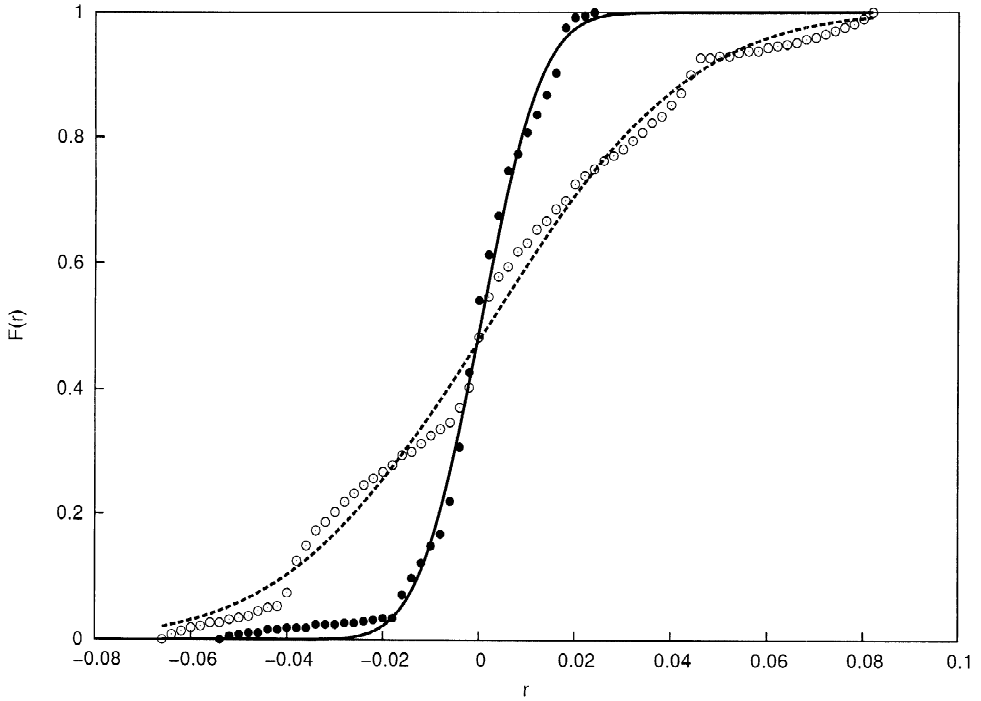

Fig. 12. Test for normality on the residual errors. Errors for the 3-parameter (open circles) and the 4-parameter (full circles) Langmuir isotherm fittings are plotted.

We must recall, however that the Langmuir–Freundlich isotherm is not really adequate for the calculation of chromatograms because of the infinite initial slope of the isotherm. This major drawback is clearly shown now when we estimate the isotherm via the shape of the elution bands.

The FSSR for the Tóth isotherm is twice that for the biLangmuir model and half of that for the Langmuir and the Langmuir–Freundlich isotherm. Both ratios are significant. Note that the Tóth isotherm parameters found here are completely different from those determined by frontal analysis. This is probably due to the difference between the two methods in the translation and propagation of the errors.

6. Conclusions

The competitive adsorption isotherms of two enantiomers can be derived from the elution profile of one overloaded injection of the racemic mixture, carried out under such conditions that the resolution of the two enantiomers is modest. Since enantiomers have identical UV response factors, the detected absorbance signal can easily be transformed into a concentration profile with a simple calibration. Note that excellent results were obtained although the sum of the two solutions of the chromatographic problem, $C_1(t) + C_2(t)$, are used in the solution of the inverse problem, not the two separate solutions, $C_1(t)$ and $C_2(t)$ as it would be necessary in the case of a mixture of other components than enantiomers. The inverse method offers a rather quick method of isotherm determination, with minimal sample and solvent use. It is important, however, to choose the proper isotherm model. A good optimization program will always give a set of best values of the parameters. This does not guarantee that the result makes physical sense. It is important to select a set of suitable candidates for the isotherm model, from

which the best-fit isotherm can be selected on the basis of the results of statistical tests.

In the case studied here, that of the adsorption of 1-phenyl-1-propanol on cellulose tribenzoate, the biLangmuir model is the most appropriate to account for the band profiles recorded. This result is not surprising, because this model is the only one in the set studied to exhibit a bimodal adsorption energy distribution. The Langmuir isotherm corresponds to a homogeneous adsorbent, which a chiral stationary phase is certainly not. The Tóth and the Langmuir–Freundlich isotherms describe heterogeneous surfaces with a unimodal adsorption energy distribution. Although the tail of this distribution can account for a weak second mode, the fit cannot be perfect. From the saturation capacity obtained for the biLangmuir isotherm, we can calculate the loading factors of the four injections made as 0.48, 0.93, 1.67, and 2.21%, respectively. The FSSR data reveal that the biLangmuir model fits significantly better the profile with the smallest value of the loading factor than the Langmuir model, with $F = 1.54$. At higher (but still moderate) values of the loading factor, the F-ratio is between 5 and 6, indicating the superiority of the biLangmuir model for the system studied.

Our results indicate that the inverse method gives rather accurate estimates of the isotherm parameters up to the maximum elution concentration of the bands used for the calculations. It is moderately accurate from the maximum elution concentration up to the injected concentration. This observation is obviously explained by the behavior of chromatographic bands during their elution. When the sample is injected, its concentration decreases rapidly at the beginning of the column and more progressively later. Although the concentration range between the sample concentration and the maximum concentration of the band is involved in the numerical integration of the mass-balance equation, the peak that we detect and use in the calculations is recorded at the column end, where the sample is significantly dilute. Note that the density of the dots in Fig. 4 increases continuously from the injected concentration toward the maximum elution concentration. The darkest regions in Fig. 4 show which concentration range is sampled most when the isotherms are estimated with the inverse method.

Therefore, we suggest that to minimize dilution, experiments should be carried out with short columns when isotherm determination with the inverse method is the goal. A general procedure implementing this approach is under investigation.

Acknowledgements

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